A NEUROHISTOLOGICAL STUDY OF THE REGENERATION OF NERVES IN A COLOSTOMY AND IN ADHESIONS BETWEEN OVARY AND INTESTINAL WALL

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A NEUROHISTOLOGICAL STUDY OF THE REGENERATION OF NERVES IN A COLOSTOMY AND IN ADHESIONS BETWEEN OVARY AND INTESTINAL WALL

by

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1. INTRODUCTION

OTSU (1953) of our clinic studied the regenenated nerves in the anastmosed part between the stomach and the jejunum in material taken from a patient who had been suffering for one year from the dumping syndrome after gastric resection for gastric ulcer. He found numerous regenerated fibers in the granulation tissue along the suture of the gastro-jejunostomy. This finding led him to the conclusion that the dumping syndrome and the pain which occur for a long period after gastrectomy may be attributed to hypersensitivity of the anastmosed part.

In addition to the dumping syndrome, surgeons have many undesirable experiences of patients who complain of abdominal pain, nausea, constipation, etc. In many cases these symptoms are considered to be due to postoperaive adhesions.

The author, therefore, studied the regeneration of nerve fibers in the adhesions between a viscus and the abdominal wall and between two viscera.

As material for studying the former, the author used tissues of a colostomy, while for the latter adhesions between ovary and intestine.

2. MATERIALS AND METHODS

(1) Adult dogs are used for the experiments. Laparotomy was done on the left side of the abdomen and a colostomy was made there with the sigmoid.

(a) The animals were sacrificed 15-60 days after the operation and the colostomy was removed including a small zone of the surrounding skin.

(b) For studying the origin of the regenerated nerve fibers in adhesions, an operation was done again on some animals 1 month after forming the colostomy. A circular section of the abdominal wall was removed 1 cm around the colostomy and the wound sutured immediately. The animal was sacrificed 2-5 days after the second operation and material was removed as in case (a).

(2) In some animals, the ovary was sutured to the ileum or sigmoid to make an artificial adhesion. For this purpose the wall of the ovary was exposed from the peritoneal capsule and a part of the serous membrance of the intestinal wall was removed before suturing to make a tight adhesion between these organs.

a) 14～50 days after the operation the adherent part was removed for micro-

scopic observation.

b) In some animals, 4 weeks after the operation, a circular section of the intestinal wall was removed several milimeters around the adhesion. From 2 to 5 days after this procedure the ovary with adherent flap of intestinal wall was removed for investigation.

These materials were fixed in 10% neutral formol solution immediately after excision, frozen and sliced in thicknesses of 30～40μ.

The axis cylinder of the nerve was stained with SETO or SUZUKI's modification of Bielschowsky's silver impregnation method and the myelin sheath with Ehrlich's acid hematoxylin method.

3. MICROSCOPIC OBSERVATION

1) Neurohistological studies of the colostomy

a) Fifteen days after constructing the colostomy, a marked proliferation of granulation tissue was observed in the adhesion between the intestinal wall and the skin, especially in the area of the colostomy.

In the granulation tissue, regenerated nerve bundles run along newly formed capillary blood vessels. The nerve bundles, consisting of a few unmyelinated fibers, follow rather straight course in the granulation. Some nerve bundles form a large network after irregular arborizations (Fig. 1 & 2).

The proliferation of these regenerated nerve fibers shows a peak about 30 days after the operation. At this stage, a great number of the nerve bundles run in the granulation, each of which consist of many unmyelinated nerve fibers. They look as though they were drawn with a paint brush (Fig. 3 & 4).

This finding is very similar to OTSU's description of granulation tissue developing along the suture line of gastrojejunostomies. With this finding he explained the cause of the dumping syndrome as local hypersensitivity.

No normal autonomic nervous network was observed at this stage.

A great number of nerve fibers were always found just under the regenerated epithelium of the skin in the adhesion. They coursed in a disorderly way under the epithelium, but they did not enter the epidermis. These nerve fibers communicated with Auerdach's plexus (Fig. 5 & 6).

On a specimen taken 31 days after operation, thick nerve fibers appeared among the regenerated fine nerve fibers (Fig. 7).

These thick nerve fibers were presumed to be myelinated sensory fibers.

In a specimen taken 17 days after operation, nerve fibers of various calibers showed marked proliferation in the subcutaneous tissue (Fig. 8).

In the same specimen, abnormally thick nerve fibers were found in the granulation tissue between the abdominal wall muscles and the intestinal wall. They appeared to grow out from the abdominal wall and did not enter the intestinal wall (Fig. 9).

In a specimen taken 30 days after operation, numerous nerve fibers ran from the muscular layer of the intestine to the granulation tissue through the defect of

the serous membrane (Fig. 10).

The proliferation of regenerated nerve fibers was found usually only in parts of the adhesion covered by granulation tissue. Loosely adhering portions with poorly developed granulation tissue did not show abnormal proliferation of regenerated nerve fibers.

The abnormally proliferated regenerated nerve fibers were not found in a specimen taken 45 days after the operation. Similarly they had already disappeared in a specimen excised 60 days after operation. In their stead, there were found a few normal nerve fibers and normal autonomic nervous syncytia (Fig. 11 & 12).

b) Next the author studied specimens removed 2～5 days after a circular section of abdominal wall, 1 cm around the opening of the colostomy and the wound had been immediately sutured.

In the specimens taken 2 days after the operation only normal nerve fibers were observed in the granulation tissue as well as in the skin (Fig. 13).

In the specimens taken 5 days after operation, the nerve fibers in the skin surrounding the colostomy had already almost disappeared, but normal nerve fibers were found in the granulation tissue of the adhesion and they were followed to the area under the regenerated epithelium (Fig. 14 & 15).

The origin of these normal nerve fibers must be in the intestinal wall and not in the abdominal wall.

2) Observation of adhesions between the ovary and intestine

a) In the specimens taken 33 & 45 days after operation the granulation tissue in the adhesion was poorly developed proliferation of regenerated nerve fibers was observed only in a limited area around the sutures, but they could not be pursued to the ovarian tissue or to the intestinal wall (Fig. 16 & 17).

b) Observation of specimens removed 5 days after excision of the intestinal wall 5 mm around the adhesion. Many of these specimens showed a marked inflammatory change. Normal nerve fibers were found in the muscle layer in the intestinal flap, which extended into the granulation tissue of the adhesion accompanied by regenerated capillary blood vessels.

They presented no features of degeneration and never entered ovarian tissue beyond the bordering granulation (Fig. 18).

The nerve cells in Auerbach's plexus showed slight degeneration such as hyperchromatic protoplasm and marginal dislocation of the nuclei but there was no sign that any of them were already dead (Fid. 19).

Generally there were no myelinated fibers in granulation tissue of adhesion; Ehrlich's hematoxylin method always showed negative results.

4. DISCUSSION

The peripheral nerves are known to have a relative great capacity for regeneration and the regenerated nerve fibers extend along the capillary bood vessels in the granulation tissue.

Maekawa (1921) reported that regenerating nerve fibers are unmyelinated in

the early stage, but after the complete growth of the axons the myelin sheath is formed around them gradually. ARIMOTO (1928) found numerous regenerated nerve fibers in the granulation tissue developing after sectioning the skin of dogs and pigeons.

MORIKAWA (1953) observed regenerated nerve fibers in the recanalisation tissue of blood vessels 2 or 3 months after organic thrombosis of the common carotid artery.

KNOTH (1955) found some regenerated nerves in the granulation tissue of wounds of human beings.

By means of electron microscopy B. B. GEREN (1954) reported an interesting finding in the formation from the Schwann cell surface of myelin in the peripheral nerves of chick embryos. During development, naked axon comes to be embedded in a mesaxon which is made of infoldings of the Schwann cell surface. Some axons are surrounded by only one layer of mesaxon, while others are wrapped in spirals with many layers of mesaxon forming lamellae around the axon. The former are unmylinated fibers and the latter are myelinated. The same process was noted by him during regeneration of peripheral nerves. These facts point out that in the regeneration of nerve fibers, the axon and the Schwann cell grow first, the unmyelinated nerve fiber second and the myelinated fiber last.

As for the developement of Schwann cells, R. CAESAR et al. supposes that they never form syncytia but are always separated as individual cells with cell membranes. Therefore the developement of Schwann cells along the new axon may not be an extension of the syncytium but may be due to the proliferation of Schwann cells.

In this study, the author observed the regenerated nerve fibers in the granulation tissue of the adhesion between the intestine and the abdominal wall, and between the intestine and the ovary for 2～8 weeks.

The developement of axons was found first, but Schwann cells did not follow them. Probably Schwann cells were covered with numerous axons in the silver impregnated specimens.

Myelinated fibers were not observed by Ehrlich's acid hematoxylin method in the regeneration tissue throughout the period of the author's investigation for 60 days. In other words, all of the nerve fibers in the granulation tissue were unmyelinated.

Concerning the origin of nerve fibers in the granulation tissue, the author could not find any degenerated fiber 2～5 days after circular section of the surrounding abdominal wall. This does not suggest that some nerve fibers come from the abdominal wall to the granulation tissue.

On the other hand, as figs. 13, 14 & 15 show, some thick nerve fibers with no sign of degeneration were found in the granulation tissue. Therefore, these nerve fibers must have developed from the intestinal side.

The author considers that most of the nerve fibers found in the granulation tissue were of intestinal origin, though it cannot be denied that there were a very

few fibers arising from the abdominal wall.

The unmyelinated regenerated sensory fibers in the granulation tissue are believed to evoke a very disagreeable sensation.

These unmylinated nerve fibers usually reach their maximum number after about 30 days and then gradually decrease. Therefore adhesions following laparotomy may usually cause a disagreeable sensation on stimulation of these nerve fibers, but this will gradually recover a few months after the operation.

However, Otsu found these unmyelinated fibers in the granulation tissue of the anastomosis between the stomach and the small intestine one year after the operation in a patient who complained of postoperative dumping syndrome.

Okawa found a great number of unmyelinated fibers in the granulation tissue of the skin in a patient who had been suffering from causalgia.

These roports suggest that in some pathologic cases these regenerated unmyelinated fibers can exist for a long period of time and cause pain, dyskinesia of the intestine or causalgia.

In this study the author concluded that most of these nerve fibers in the granulation tissue between the abdominal wall and the intestine are of intestinal origin. Therefore, he cannot conceive that the intestinal wall adhering to the abdominal wall could get its sensitivity from the abdominal wall but mechanical stimulation stretching the adhesion, or chemical stimuli such as inflammation substances produced in the adhesion may cause dyskinesis of the intestine through unmyelinated sensory nerve fibers in the granulation tissue.

These findings may possibly explain the cause of the disagreeable sensation and dysfunction of the intestine resulting from adhesions following laparotomy, which usually disappear within few months after the operation except for rare instances of persistent dyskinesis.

5. SUMMARY

Using adult dogs, the author constructed colostomies and studied the neurohistology in the granulation tissue developed between the abdominal wall and the intestine. The results were as follows.

(1) Unmyelinated regenerated nerve fibers proliferate in the granulation tissue between the abdominal wall and the intestine, their number reaching a peak 1 month after the operation and then gradually decreasing. Two months after the operation, these abnormal fine regenerated fibers have already disappeared in the granulation tissue and only normal nerve fibers are observed.

(2) No degenerated nerve fibers are found in the specimens, removed 2~5 days after a circular section of abdominal wall surrounding the colostomy was removed and the wound sutured immediately. This suggests that most of the regenerated nerve fibers grown in the granulation tissue are of intestinal origin.

(3) Only a very few regenerated nerve fibers are observed in the granulation tissue developed between the ovary and intestine.

(4) The transient proliferation of the regenerated unmyelinated fibers usually

disappears within 2 months, but rarely it persist for a longer period of time and causes abdominal neurosis (ARAKI) or dyskinesis of the intestine. It is very difficult to conceive that the intestine will get the same sensitivity as the abdominal wall by adhering to it.

The author is greatly indebted to Assist. Prof. CH. KIMURA for his kind advice during the course of this study.

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Description of Figures

1) Regenerated nerve fibers in granulation tissue at site of incision of colostomy 15 days after operation. ×400 B(Dog)
2) Regenerated nerve fibers in granulation tissue at the point of suture, forming a large network. 15 days after the operation of artificial anus. ×400(Dog)
3) Regenerated nerve fibers in granulation tissue at the site of incision of the colostomy. ×400(Dog)
4) Same as Fig. 13.
5) Regenerated nerve fibers beneath the regenerated epidermis at the point of suture. 15 days after colostomy. ×400 (Dog)

6) Same as Fig. 5.
7) Regenerated thick nerve fibers beneath the regenerated epidermis at the point of suture. 31 days after colostomy. ×400 (Dog)
8) Regenerated nerve fibers in the subcutaneous tissue. 17 days after the operation. ×400 (Dog)
9) Regenerated abnormal thick nerve fibers in the granulation tissue between the abdominal wall muscles and the intestinal wall. ×400 (Dog)
10) Regenerated nerve fibers running from the muscular layer of the intestine into the granulation tissue. ×400 (Dog)
11) Normal autonomic nervous syncytia observed at the point of suture of the sigmoid and the hypoderm. 60 days after the operation. ×400 (Dog)
12) Normal nerve fibers running from the submucous tissue into the regenerated sucutaneous tissue. ×600 (Dog)
13) Nondegenerated nerve fibers beneath the regenerated epidermis. 2 days after circular section around colostomy made 30 days before. ×400 (Dog)
14) Nondegenerated nerve fibers observed beneath the regenerted epidermis. 3 days after the same operation as Fig. 13.
15) Nondegenerated nerve fibers at the point of suture of the muscular tissue of the abdominal wall and the intestine. 5 days after the same operation as Fig. 13. ×400 (Dog)
16) Regenerated nerve fibers in the granulation tissue of the adhesion. 33 days after the operation. A silk thread can be seen in the right upper corner. ×400 (Dog)
17) Regenerated nerve fiber in adhesion. 45 days after operation. ×400 (Dog)
18) Nondegenerated nerve fiber in the muscule layer in the flap of descending colon. 5 days after section.
19) The nerve cells in Auerbach's plexus showing only slight degeneration. 5 days after the operation.

和文抄録

人工肛門及び内臓癒着部に於ける再生神経に関する組織学的研究

京都大学医学部外科学教室第2講座 (指導：青柳安誠教授)

杉浦純宦

成犬を用い，人工肛門及び卵巣-腸壁癒着部を作りBielschowsky氏神経鍍銀法の瀬戸氏変法，鈴木氏変法及びEhrlich氏神経髄鞘染色を用いて，癒着部肉芽組織に於ける再生神経について，次の結果を得た．

1) 人工肛門の腹壁と腸壁の癒着部肉芽組織に無髄神経線維が再生増殖し，術後1ヵ月で数的に最高に達し，以後減少して術後2ヵ月では異常再生神経は見られず，正常な神経線維のみが認められる．

2) 人工肛門造設後1ヵ月経て，腹壁を人工肛門の周囲で輪状に切断し，再縫合して2～5日後摘出した標本では癒着部の神経線維に変性像が認められない．この神経線維の大部分は腸壁より再生していると考えられる．

3) 卵巣と腸壁の癒着部肉芽組織には再生神経線維が僅かに認められる．

4) 癒着部に再生した無髄神経線維の過剰増殖は一時的であつて，通常2ヵ月以内に消失するが永続的ならば異常であつて，術後性腹部神経症 (荒木) 或いは腸管の運動障害の一つの原因となり得る事を考察した．人工肛門に於て癒着に依つて腸壁が腹壁と同様な知覚を得る様になるとは考え難い．

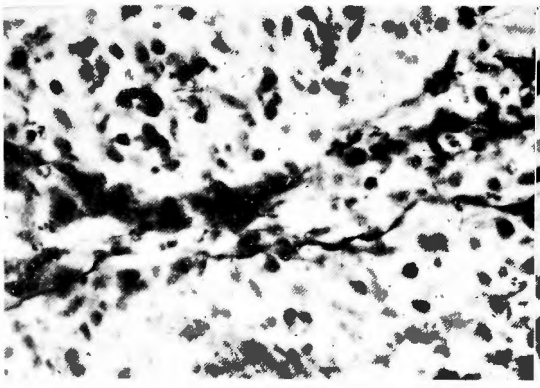
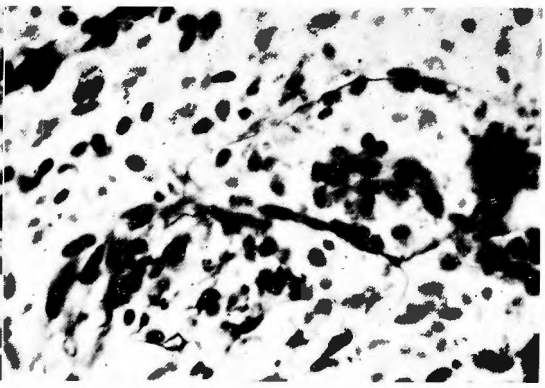


Fig. 1



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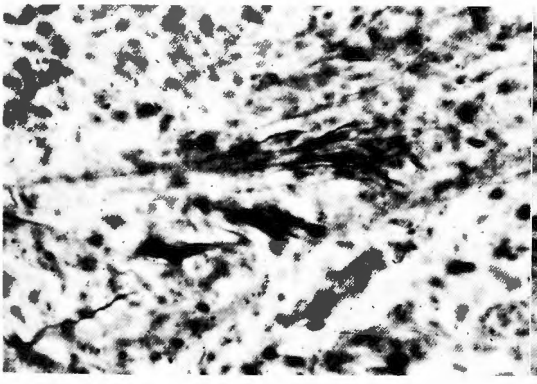

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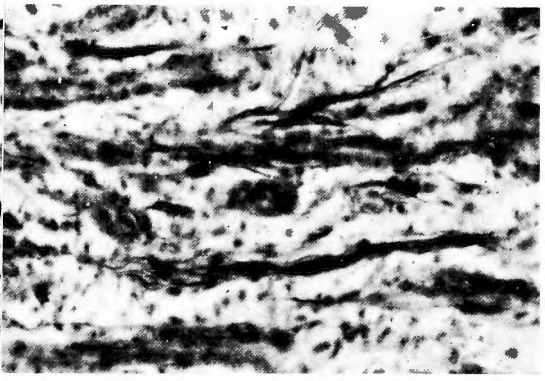

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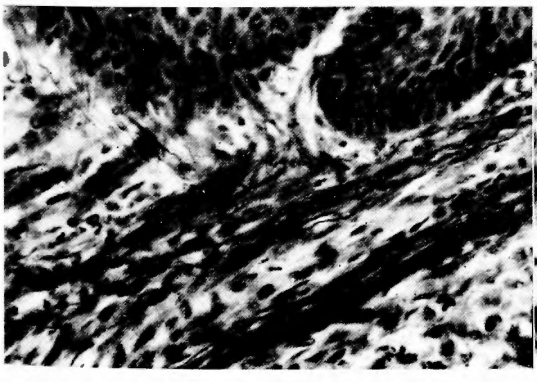

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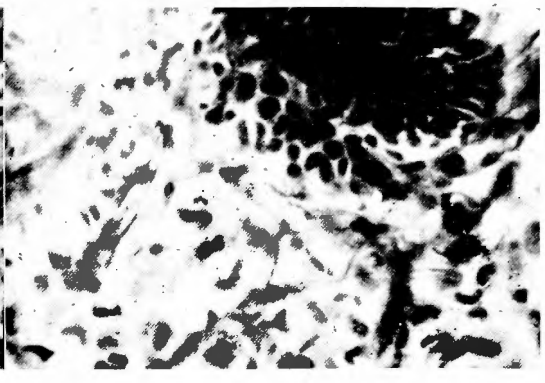

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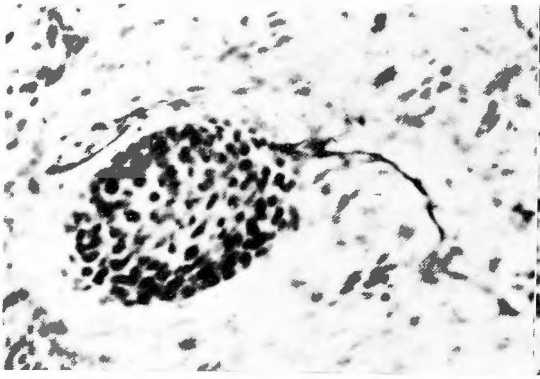

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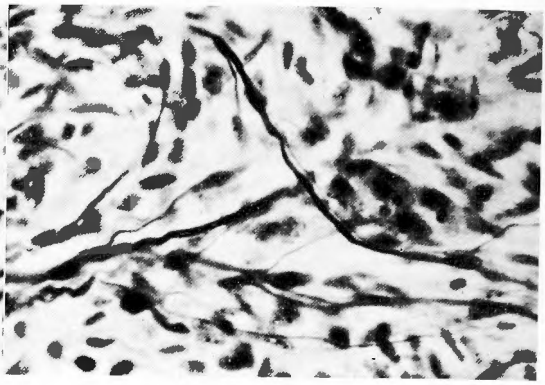
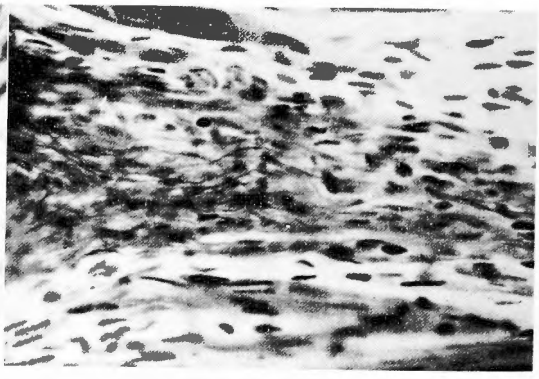


Fig. 8

Fig. 9



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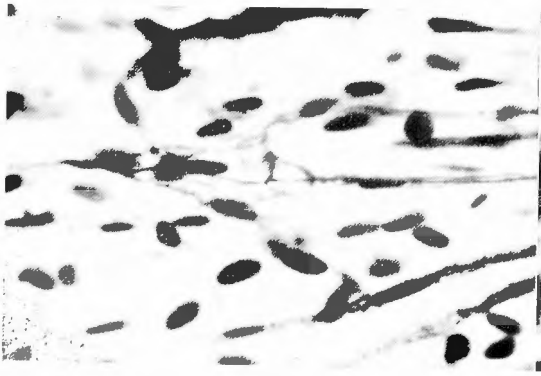

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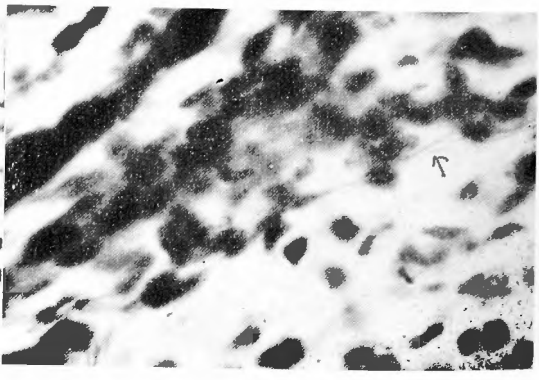

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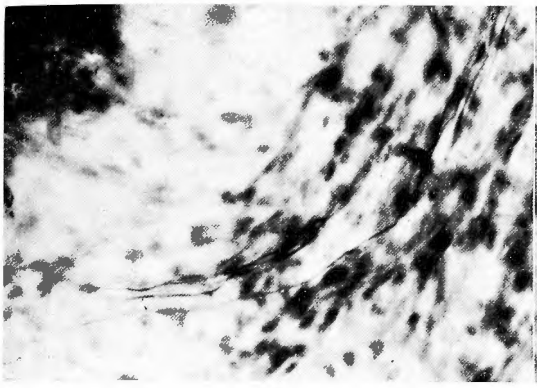


Fig. 13

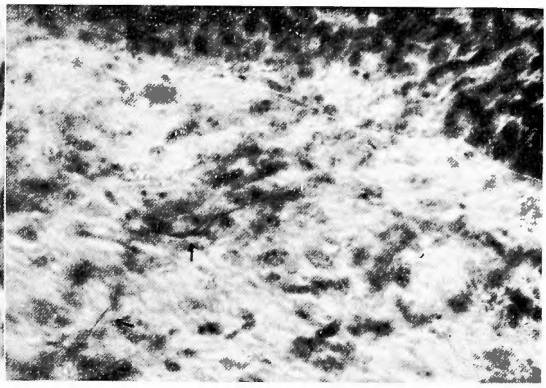
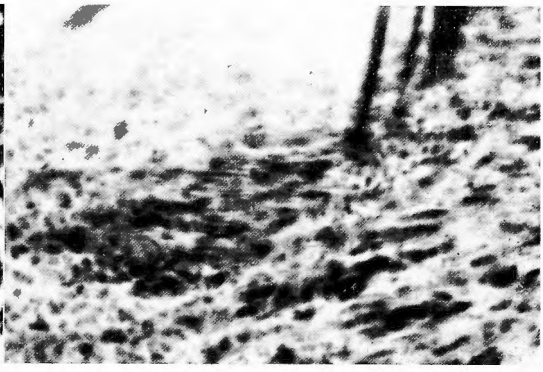


Fig. 14

Fig. 15



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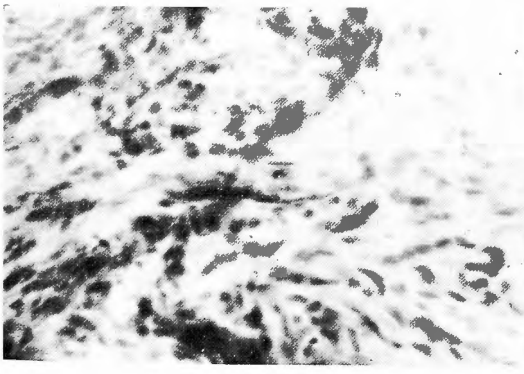

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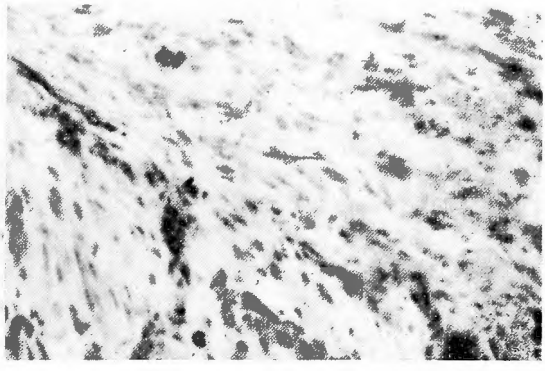

Fig. 18

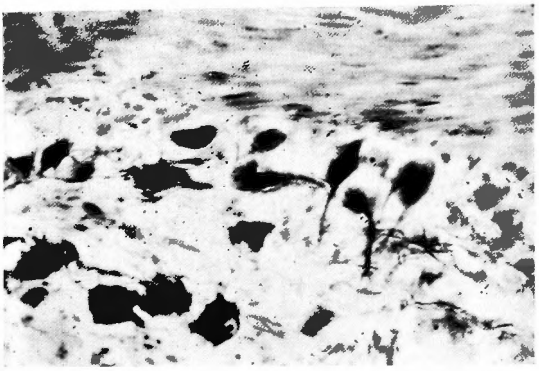

Fig. 19

CORRECTION OF THE MISPRINTS IN SUGIURA'S ORIGINAL PUBLISHED IN THE "ARCHIV FÜR JAPANISCHE CHIRURGIE" VOL. 27 ; No. 3 P.597—608, 1958

LOCATION PAGE	OF MISPRINT	LETTERS MISPRINTED	LETTERS CORRECTED
600	+ 1	findneither HURREII	find neither HURRELL
601	+16	cp &	c. p. s.
	−15	metioned	mentioned
604	Fig. 8.	hone	bone
	Fig. 10.	10 Nerve	Nerve
	Fig. 12.	Finene rve	Fine nerve
605	Fig. 16.	Narrow	marrow
606	Fig. 22.	femur	tibia
	Fig. 23.	femur	tibia
607	+10	RFERENCES	REFERENCES
	Right +16	knochenmark	Knochenmark
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	−18	af the	of the
	Left +18	Roshash, D. C.	Rpsbash, D. O.
	+21	C.	O.
	+22	befinlchen	befindlichen
	−15	1-2,	20,
	−10	Cbservation	Observation
	− 6	Pre.	Pref.
	− 4	Cbservation	Observation
608	+ 4	純 崖	純 宦
	Right + 5	BIELSCHOSHY	BIELSSCHOWSKY
	Left + 7	D. G. HURREII	D. J. HURRELL